PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. 615-25A

Anticipated Classification of this application:

Class 435 Subclass 29

Prior application:

Examiner: R. Schwadron

Art Unit: 1644

Commissioner of Patents and Trademarks

Washington, D.C. 20231

FILING UNDER 37 CFR 1.60(b)

WARNING: *A c-i-p (continuation-in-part) cannot be filed under 37 CFR 1.60.*

WARNING: *Filing under 37 CFR 1.60 is permitted only if filed by the same or less than all the inventors named in the prior application.*

WARNING: *The filing of an application as the United States stage of an International Application requires an oath or declaration. 37 CFR 1.61(a)(4).*

WARNING: *The claims of this new application may be finally rejected in the first Office action where all claims of the new application are drawn to the same invention claimed in the earlier application and would have been properly finally rejected on the grounds or art of record in the next Office action if they had been entered in the earlier application. MPEP § 706.07(b).*

This is a request for filing a

☐ Continuation

☒ Divisional

application under 37 CFR 1.60, of pending prior application

serial no. 08/ 446,760 filed on May 26, 1995
(*date*)

of Birgit A. HELM, Anne P.M. WILSON, Denise MOREIRA-MACHADO, Christine PULLAR and Andrew CAMP (*inventor(s)*)

for ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS
(*title of invention*)

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this 37 CFR 1.60 request and the documents referred to as attached therein are being deposited with the United States Postal Service on this date 8/12/98 in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10, Mailing Label Number EM425193617 US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

THOMAS M. GALGANO, ESQ.
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing. (37 CFR 1.10(b)).

NOTE: 37 CFR 1.60 permits the omission of a declaration only if the prior application was complete as set forth in 37 CFR 1.51(a), namely, the prior application comprised at least (1) a specification, including a claim or claims; (2) a declaration; (3) drawings when necessary; and (4) the prescribed filing fee. Accordingly, as presently worded, 37 CFR 1.60 does not permit this procedure to be used where the prior application is pending but only the processing and retention fee required by 37 CFR 1.21(l) is paid or where the declaration was not filed.

1. Copy of Prior Application as Filed Which is Attached

NOTE: Under 37 CFR 1.60 practice signing and execution of the application by the applicant may be omitted provided the copy is supplied by and accompanied by a statement by the applicant or his or her attorney or agent that the application papers comprise a true copy of the prior application as filed and that no amendments referred to in the declaration filed to complete the prior application introduced new matter therein.

NOTE: This statement need not be verified if made by an attorney registered to practice before the PTO. (37 CFR 1.60(b)).

☒ I hereby verify that the attached papers are a true copy of what is shown in my records to be the above identified prior application, including the oath or declaration originally filed (37 CFR 1.60).

The copy of the papers of prior application *as filed* which are attached are as follows:

☒ 19 page(s) of specification

☒ 4 page(s) of claims

☒ 1 page(s) of abstract

☐ _____ sheet(s) of drawing

(Also complete part 6 below if drawings are to be transferred)

☐ _____ pages of declaration and power of attorney

If the copy of the declaration being filed does not show applicant's signature indicate thereon that it was signed and complete the following:

☐ in accordance with the indication required by 37 CFR 60(b) my records reflect that the original signed declaration showing applicant's signature was filed on _____

☐ the amendment referred to in the declaration filed to complete the prior application and I hereby state, in accordance with the requirements of 37 CFR 1.60(b), that this amendment did not introduce new matter therein.

2. Amendments

WARNING: *"The claim of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds or art of record in the next Office action if they had been entered in the earlier application." MPEP § 706.07(b).*

☒ Cancel in this application original claims 1-15, 25-30 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered **original** claim in the prior application.)

NOTE: Only amendments reducing the number of claims or adding a reference to the prior application (Rule 1.78(a)) will be entered before calculating the filing fee and granting the filing date. 37 CFR 1.60(b).

NOTE: "When filing under Rule 1.60 retain at least one original claim from the patent application to assure a complete application." Notice of March 3, 1986 (1064 O.G. 37-38).

3. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

Note: Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary).

(check the next item, if applicable)

☐ There is provided herewith a Petition To Suspend Prosecution For The Time Necessary to File An Amendment (New Application Filed Concurrently).

4. Fee Calculation (37 CFR 1.16)

CLAIMS AS FILED

	Number filed		Number Extra		Rate	Basic Fee
						$790.00
Total Claims	9	−20=	0	×	$22.00	0
Independent Claims (37 CFR 1.16(b))	2	−3=	0	×	$ 82.00	0
Multiple dependent claim(s), if any (37 CFR 1.16(d))				×	$ 260.00	

☐ Fee for extra claims is not being paid at this time. (37 CFR 1.16(d))

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ 790.00

5. Small Entity Status

☒ A verified statement that this filing is by a small entity:

☐ is attached

☒ has been filed in the parent application and such status is still proper and desired (37 CFR 1.28(a))

Filing Fee Calculation (50% of above) $ 395.00

NOTE: Any excess of the full fee paid will be refunded if a verified statement is filed within 2 months of the date of timely payment of a full fee then the excess fee paid will be refunded on request. 37 CFR 1.28(a).

NOTE: 37 CFR 1.28(a), last sentence states: "Applications filed under § 1.60 or § 1.62 of this part must include a reference to a verified statement in a parent application if status as a small entity is still proper and desired."

6. Drawings

WARNING: Do not check the following box if prior case is not to be abandoned.

☐ Transfer the drawings from the prior application to this application and, subject to item 17 below, abandon said prior application as of the filing date accorded this application. A duplicate copy of this request is enclosed for filing in the prior application file. *(May only be used if signed by (1) applicant, (2) assignee of record or (3) attorney or agent of record authorized by 37 CFR 1.138 and before payment of issue fee.)*

NOTE: "A registered attorney or agent acting under the provisions of § 1.34(a), or of record, may also expressly abandon a prior application as of the filing date granted to a continuing application when filing such a continuing application." 37 CFR 1.138.

☐ Transfer the following sheet(s) of drawing from the prior application to this application _____

NOTE: Transferred sheets must be cancelled in prior application. 37 CFR 1.88.

☐ A copy of the amendment cancelling these sheets of drawing in the prior application is attached.

☒ New drawings are enclosed

☒ formal

☐ informal

***WARNING:** DO NOT submit original drawings. A high quality copy of drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards of § 1.84. If corrections to the drawings are necessary, they should be made to the original drawings and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. Comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1090 O.G. 57-62).*

NOTE: "Identifying indicia such as the serial number, group art unit, title of the inventor, attorney's docket number, inventor's name, number of sheets, etc. not to exceed 2¼ inches (7.0 cm.) in width may be placed in a centered location between the side edges within three fourths inch (19.1 mm.) of the top edge. Either this marking technique on the front of the drawing or the placement, although not preferred, of this information and the title of the invention on the back of the drawings is acceptable." Proposed 37 CFR 1.84(1). Notice of March 9, 1988 (1090 O.G. 57-62).

7. Priority—35 U.S.C. 119

☒ Priority of application serial no. XXX 92 249 56.4 filed on November 28, 1992 in Great Britain (country) is claimed under 35 U.S.C. 119.

☒ The certified copy has been filed in prior U.S. application serial no. 08/446,760 on 7-17-95

☐ The certified copy will follow.

8. Relate Back—35 U.S.C. 120

☒ Amend the specification by inserting before the first line the sentence:

"This is a

☐ continuation

☒ divisional

of copending application(s)

☒ Serial number 08/ 446,760 filed on 7/21/95" which, in turn, is based on

☒ International Application PCT/GB93/02430 filed on November 25, 1993 and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application which entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application which designated the U.S.

9. Inventorship Statement

NOTE: If the continuation or divisional application is filed by less than all the inventors named in the prior application a statement must accompany the application when filed requesting deletion of the names of the person or persons who are not inventors of the invention being claimed in the continuation or divisional application. 37 CFR 1.60(b) [emphasis added].

(complete appropriate items (a) and (b))

(a) With respect to the prior copending U.S. application from which this application claims benefit under 35 USC 120 the inventor(s) in this application is (are):

(complete applicable item below)

☒ the same

☐ less than those named in the prior application and it is requested that the following inventor(s) identified above for the prior application be deleted:

_____

(Type name(s) of inventor(s) to be deleted)

(b) The inventorship for all the claims in this application are

☒ the same

☐ not the same, and an explanation, including the ownership of the various claims at the time the last claimed invention was made, is submitted.

10. Assignment

☒ The prior application is assigned of record to EURO/DPC Limited

☐ an assignment of the invention to _____

_____

is attached

11. Fee Payment Being Made At This Time

☐ Not Enclosed

☐ No filing fee is submitted. *(This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently).*

☒ Enclosed

☒ basic filing fee $ 395.00

☐ recording assignment ($8.00; 37 CFR 1.21(h)) $ _____

☐ processing and retention fee ($120.00; 37 CFR 1.53(d) and 1.21(l)) $ _____

NOTE: *37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(d) and this, as well as the changes to 37 CFR 1.53 and 1.78 indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid or else the processing and retention fee of § 1.21(l) must be paid within 1 year from notification under § 53(d).*

Total fees enclosed $ 395.00

12. Method of Payment of Fees

☒ enclosed is a check in the amount of $ 395.00

☐ charge Account No. _____ in the amount of $_____
A duplicate of this request is attached.

NOTE: *Fees should be itemized in such a manner that is clear for which purpose the fees are paid. 37 CFR 1.22(b).*

13. Authorization To Charge Additional Fees

WARNING: *If no fees are being paid on filing do not complete this item.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claim charges are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees which may be required by this paper and during the entire pendency of the application to Account No. 07-0130

☒ 37 CFR 1.16 (a), (f) or (g) (filing fees)

☒ 37 CFR 1.16 (b), (c) and (d) (presentation of extra claims)

NOTE: *Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)) it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.*

☒ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under § 1.136(a) this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 CFR 1.136(a) is to no avail unless a request or petition for extension is filed." [emphasis added]. Notice of November 5, 1985 (1060 O.G. 27).*

☐ 37 CFR 1.18 (issue fee at or before mailing Notice of Allowance, pursuant to 37 CFR 1.311(b)).

NOTE: *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b)).*

NOTE: *37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying or at the time of paying . . . issue fee." From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

14. Power of Attorney

☒ The power of attorney in the prior application is to

Thomas M. Galgano, Reg. NO. 27,638 / Daniel P. Burke, Reg. No. 30,735

Attorney — Reg. No.

a. ☐ The power appears in the original papers in the prior application.

b. ☒ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. ☐ A new power has been executed and is attached.

d. ☒ Address all future communications to

Galgano & Burke
U.S. Federal Courthouse Building
300 Rabro Drive - Suite 135
Hauppauge, New York 11788

(Item d may only be completed by applicant, or attorney or agent of record)

15. Maintenance of Copendency of Prior Application

*(This item must be completed and the papers filed in the **prior** application if the period set in the prior application has run)*

☐ A petition, fee and response has been filed to extend the term in the pending prior application until _____

*NOTE: The PTO finds it useful if a **copy** of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the Continuation Application. Notice of November 5, 1985 (1060 O.G. 27).*

☐ A **copy** of the petition for extension of time in the prior application is attached.

16. Conditional Petition for Extension of Time in Prior Application

(complete this item and file conditional petition in the prior application if previous item not applicable)

☐ a conditional petition for extension of time is being filed in the pending parent application.

*NOTE: The PTO finds it useful if a **copy** of the petition filed in the prior application extending the term for response is filed with the paper constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).*

☐ A **copy** of the conditional petition for extension of time in the prior application is attached.

17. Abandonment of Prior Application (if applicable)

***WARNING:** (Do not complete this item if the application being filed is a divisional of the prior application which is not being abandoned)*

NOTE: "A registered attorney or agent acting under the provisions of § 1.34(a), or of record, may also expressly abandon a prior application as of the filing date granted to a continuing application when filing such a continuing application." 37 CFR 1.138.

☐ Please abandon the prior application at a time while the prior application is pending or when the petition for extension of time or to revive in that application is granted and when this application is granted a filing date so as to make this application copending with said prior application.

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

THOMAS M. GALGANO, ESQ.
Type or print name of person signing

8/12/98
Date

Signature

GALGANO & BURKE
300 Rabro Drive
Suite 135
P.O. Address of Signatory

Hauppauge, NY 11788

Tel. No.: (516) 582-6161

Reg. No. 27,638
(if applicable)

- ☐ Inventor
- ☐ Assignee of complete interest
- ☐ Person authorized to sign on behalf of assignee
- ☒ Attorney or agent of record
- ☐ Filed under Rule 34(a)

(Complete the following if applicable)

EURO/DPC LIMITED
Type name of assignee

Glyn Rhonwy, Llanberis
Address of assignee

Caernarfon, Gwynedd

LL55 4EL, Great Britain
Title of person authorized to sign on behalf of assignee

Assignment recorded in PTO on 7/21/95

Reel 7692 Frame 0267

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service, Express Mail No. EM425193617US under 37 C.F.R. 1.10 on date indicated below and is addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on August 12, 1998.

By: _____ 8/12/98
Thomas M. Galgano, Esq. Date

PATENT
DOCKET NO.: 615-25A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: BIRGIT A. HELM ET AL

FOR: ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

Preliminary to the initial Office Action, please amend the above-identified application as follows:

IN THE CLAIMS:

Please amend the claims as follows:

Claim 18, line 1, delete "or 17."

Claim 19, line 1, ", 17 or 18."

Claim 20, line 1, delete "Claims 16-19" and insert --Claim 16--.

Claim 23, line 1, delete "Claims 16-19" and insert --Claim 16--.

Claim 24, line 1, delete "Claims 16-22" and insert --Claim 22--.

REMARKS

By means of this Preliminary Amendment, the claims have been amended to remove multiple dependencies contained therein, so as to avoid the official surcharge associated therewith.

Accordingly, entry of the foregoing amendments is respectfully requested and an early and favorable action on the merits of the application is earnestly solicited.

Respectfully submitted,

BIRGIT A. HELM ET AL

Thomas M. Galgano
Registration No. 27,638
GALGANO & BURKE
Attorneys for Applicants

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C:\G&B#5\615\HELM\PRELIM.AMD

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
G01N 33/50, 33/68, A61K 31/70, 37/02

A1

(11) International Publication Number: WO 94/12876

(43) International Publication Date: 9 June 1994 (09.06.94)

(21) International Application Number: PCT/GB93/02430

(22) International Filing Date: 25 November 1993 (25.11.93)

(30) Priority Data:
9224956.4 28 November 1992 (28.11.92) GB

(71) Applicant *(for all designated States except US)*: EURO/DPC LIMITED [GB/GB]; Glyn Rhonwy, Llanberis, Caernarfon, Gwynedd LL55 4EL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HELM, Birgit, Anna [DE/GB]; 17 Oak Brook Court, Riverdale Park, Sheffield S10 3HR (GB). WILSON, Anne, Penelope, Margaret [GB/GB]; 6 Stable Mews, Mill Lane, Beaumaris, Anglesey, Gwynedd LL58 8BL (GB). MOREIRA-MACHADO, Denise [BR/GB]; 453 Springvale Road, Sheffield S10 1LQ (GB). PULLAR, Christine, Elaine [GB/GB]; 92 Heavygate Road, Walkley, Sheffield S10 1PF (GB). CAMP, Andrew [GB/GB]; 21 Grosvenor Square, Sheffield S2 4NS (GB).

(74) Agent: WILLIAM, Jones; The Crescent, 54 Blossom Street, York, North Yorkshire YO2 2AP (GB).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS

(57) Abstract

The invention relates to the use of a secretor variant cell-line expressing the alpha moiety of human IgE binding protein to determine the allergic status of a given individual. Moreover, the cell-line is also used to provide an assay system for determining the allergenicity of substances and for subsequently providing therapeutic compositions which render said substances ineffective. In addition, the invention also relates to the use of said cell-line to determine the IgE independent irritancy of substances and compositions effective for attenuating the effects of said substances.

IMPROVEMENTS RELATING TO ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS

The invention relates to a method and test system for determining an individual's sensitivity to at least one pre-selected allergen/irritant and for determining the allergenicity or inflammatory characteristics of chemicals; further, the invention also relates to a therapeutic treatment for attenuating antigen-induced or inflammatory-induced mediator response to allergenic or inflammatory stimulation, respectively.

The response of an individual sensitive or responsive to a particular allergen or irritant is typically characterized by, amongst other things, an inflammatory response. This inflammatory response is produced as a result of the release of pharmacologically active mediators from mast cells and basophils. Indeed, a number of workers have shown that the use of mast cells and/or basophils cells in allergy testing is well established, for example, the following documents demonstrate sensitisation of these cells followed by exposure to allergen and the subsequent detection of a response, US 4,559,310, EP 0 265 411, and US 3,900,558. The activation of these cells in allergic responses such as asthma and hay fever is known to be mediated by the antibody IgE. It is known that there is a high-affinity receptor for IgE (FceR1) on the surface of mast cells and basophils, and further that the aggregation of IgE-occupied receptors by antigen is responsible for the release of allergic mediators from such cells. Indeed, the use of cell-lines expressing this high affinity receptor have been used to determine the allergenicity of food additives, Japanese Journal of Toxicology and Environmental Health (1991) volume 37(5) 370-378. The FceR1 receptor is known to be made of at least three different sub units, alpha, beta and gamma. The alpha sub unit is known to bind IgE.

Investigators have successfully transfected the human FceR1 alpha sub unit into a rat mast cell-line RBL-2H3 thus producing a rat mast cell-line which is capable of expressing the human FceR1 alpha, Journal of Immunology (1992) volume 149(7) 2445-2451.

We have successfully transfected human FcerR1 alpha sub unit into a rat mast cell-line RBL-2H3 and the precise techniques which we used are described in detail in the following references: Conservation Of Signal Transduction Mechanisms Via The Human FceR1 Alpha After Transvection Into A Rat Mast Cell-Line RBL-2H3, Gilfillan, A. M., Kado-Fong, H., Wiggan, G. A., Hakimi, J., Kent, U., and Kochan J. P., Journal of Immunology 149, 2445-2451 1992; Revisiting the Basophil Degranulation Test, Wilson, A. P. M., Moreira-Machado, D., Rhodes, N., Ahmad, T. B., Pullar, C. E., and Helm, B. A., Journal of Clinical Immunoassay 16, 91-95 1993; and Human IgE mediates stimulus secretion coupling in rat basophilic leukemia cells transfected with the alpha chain of the human high-affinity receptor, Wilson, A. P. M., Pullar, C. E., Camp, A. M., and Helm, B. A., European Journal of Immunology 23, 240-244, 1993. The information and features described in these prior publications implicitly belong to the description of the invention contained in this application and thus to the content of this application. It is of note that such information and features contribute to achieving the technical aim of the invention, that is to provide a method and test system for determining sensitivity to an allergen or irritant and therapeutic treatments for attenuating same, and as such are comprising the solution of the technical problem underlying the invention which is the subject of this application. It follows that protection may be sought for such features described in these prior publications.

It has been found that this cell-line is a useful tool for understanding the fundamental steps involved in the above described response. However, we have used a high secretor variant of this cell-line to develop a method, and corresponding assay system, for determining the allergic/inflammatory status of an individual to a pre-selected allergen/irritant. Moreover, we have also used this cell-line for determining the potential allergenicity/irritancy of pre-selected chemicals. Further, the cell-line has been used to develop a therapeutic treatment for attenuating antigen-

induced or inflammatory mediator response to allergenic or irritant stimulation.

Turning to the first method and corresponding assay system, it is known that individuals sensitive to a particular allergen have in their serum allergen specific IgE molecules, these are also known as sensitizing agents. This information has been used in the past to develop skin tests for the purpose of determining the allergen sensitivity of a given individual. However, these skin tests are potentially dangerous since they can have a booster effect in an already sensitized individual and in some countries such skin tests are illegal.

We have therefore used the afore described secretor variant to develop a method which includes incubating the cell-line with serum from an individual to be tested and then challenging the cell-line with a pre-selected allergen.

It follows that if an individual's serum contains allergen specific IgE which corresponds to the allergen to be tested, the cell-line will respond in an immuno-reactive way and release pharmacologically active mediators. The detection of these mediators is therefore a means of determining whether an immunogenic reaction has taken place. This in turn signifies the allergic status of the individual.

According to a first aspect of the invention there is therefore provided a method for determining the allergic status of an individual comprising:

1. exposing a cell-line, which is a secretor variant of mast cell or basophil lineage and is transfected with a moiety capable of binding human IgE, to a sentising agent;

2. challenging the cell-line with at least one allergen; and

3. determining the release of mast cell or basophil mediators in response to said challenge.

In a preferred method of the invention the said sensitizing agent comprises human serum and more preferably human serum from the said individual, or alternatively, said sensitizing agent comprises human IgE or a functional equivalent thereof.

In a preferred method of the invention the mast cell-line is an RBL-2H3 cell-line but in any case the cell-line is transfected with a moiety capable of binding human IgE with high-affinity. Alternatively the cell-line is a secretor variant and is of mast cell or basophil lineage and is transfected with a moiety capable of binding human IgE.

Furthermore, ideally, the cell-line is first pre-incubated in a solution containing radio active, or other, marker, preferably histamine or tritiated 5-hydroxytryptamine [3H]-5HT (1uCi/ml) or 14C arachadonic acid ideally until equilibrium has taken place, then the cells are washed to remove traces of extra cellular radio activity. The cells are sensitized and challenged with allergen. At the end of the reaction the cellular environment in which the cell-line is located, that is supernatant, is assayed for the presence of radio active marker such as histamine or tritiated 5-hydroxytryptamine [3H]-5HT or 14C arachadonic acid, thus indicating the release of same from said cells.

A commercially preferred method of the invention comprises the use of a spectrophotometric or colourimetric means for determining the release of mast cell mediators. For example, the release of cell mediators such as proteases is detected by including in the cellular environment, or supernatant, a chromogen that is acted upon by said proteases in such a way that a colour change is observed. However, it is within the scope of the invention to determine protease release by using any known proteolytic assay. Alternatively, it is within the scope of the invention to determine mast cell or basophil cell activation by measuring ionic fluctuations such as mobilization of intercellular calcium or membrane potential fluctuations.

In a further preferred method of the invention, cell mediator release is determined by the use of standard antibody binding assays which specifically identify a pre-selected cell mediator.

It will be understood that the determination of a reaction can be undertaken by determining the release of any pre-selected mast cell mediator, for example, one could assay for the presence of interleukins such as interleukin 3, 4, 5 6, and 8.

According to a further feature of this aspect of the invention, there is provided an assay kit for determining the allergen sensitivity of an individual comprising:

1. a cell-line which is a secretor variant of a mast cell-line or a basophil cell-line and is transfected with a moiety capable of binding human IgE;

2. test allergen; and

3. means necessary to determine the absence or presence of an immune response.

In a preferred embodiment of the invention the cell-line is an RBL-2H3 cell-line but in any case the cell-line is transfected with the alpha-chain of the human high-affinity receptor for IgE. Alternatively, the cell-line is a secretor variant of a human mast cell-line or human basophil cell-line expressing moieties which bind human IgE with high-affinity.

Further, the preferred means comprise either an amount of radio active marker and ideally a radio active marker such as, but not limited to, tritiated 5-hydroxytryptamine or ^{14}C arachadonic acid.

Ideally the means is a chromogen or alternatively a means of measuring proteolytic activity or alternatively an assay means.

We have also developed the use of a mast cell or basophil cell-line to determine the potential allergenicity of a given chemical.

It is known that some chemicals can give rise to an inflammatory response which appears to be IgE independent. Such chemicals include bee and vespid venoms (the causative agents identified from these sources are basic proteins like mellitin and mastopran, phospholipase A, cysteine and serine protease, a number of lectins, including viral haemagluttinins that interact with carbohydrate residues on the receptor and/or IgE, and sulfiting agents (food preservatives).

Using the invention we have determined that the following chemicals elicit the release of mast cell mediators from our cell-lines and would therefore seem to give rise to an inflammatory response which is IgE independent. These chemicals include bacterial phospholipase C (B cereus), house dust mite proteins, salicylates (aspirin based drugs), latex suspensions, extract from manufactured latex products (gloves, condoms) and spermicide.

The mechanism by which these chemicals induce mast cell exocytosis in the absence of IgE has yet to be elucidated, however, the fact that this effect occurs means that a mast cell or basophil cell-line can be used to determine the potential allergenicity of chemicals by exposing the cell-line to chemicals and determining the absence or presence of a reaction by determining mast cell exocytosis or mediator release.

According to a second aspect of the invention there is therefore provided a method for determining the potential irritancy of a pre-selected substance comprising:

1. exposing a mast cell-line and/or basophil cell-line to a pre-selected amount of said substance; and

2. then determining the release of mast cell and/or basophil cell-mediators in response to said exposure.

In a preferred method of the invention the mast cell-line is a secretor variant of a cell-line such as a RBL-2H3 cell-line which may or may not be transfected with a moiety capable of binding human IgE with high-affinity. In a preferred method of the invention and especially where the substance to be tested reacts with a cell bound immunoglobulin of IgE isotype the mast cell-line or basophil cell-line is also exposed to an amount of IgE or a functional equivalent thereof. In this case, where the mast cell-line or basophil cell-line is incubated with IgE, it is preferable to use the transfected RBL-2H3 cell-line, or a human cell-line. Further, in a preferred embodiment, the mast cell-line is pre-incubated with radio active marker and ideally tritiated histamine or 5-hydroxytryptamine or 14C arachadonic acid, ideally until equilibrium has taken place, and then the cells are washed to move any extra cellular radio active or tritiated substances. The cells are then exposed to the said chemical and the cellular environment is then preferably assayed for the presence of radio active marker such as tritiated histamine thus indicating the release of same from said cells.

In a yet further preferred embodiment of the invention the cell-line is exposed to a chromogen which changes colour as a result of the presence of a reaction.

In a yet further preferred embodiment of the invention the release of cell mediators is determined using standard proteolytic assays or antibody assays as described above. Or, alternatively, by monitoring transmembrane or intracellular ion fluxes.

We have further used the cell-line to identify therapeutic compositions for attenuating antigen-induced or irritant-induced mediator response to allergenic stimulations or irritants. We have found that many of the primary

effects of both chemical- or irritant-induced mediator release from mast cell-lines and basophil cell-lines and IgE mediated, antigen-induced mediator release from mast cell-lines and basophil cell-lines can be attenuated by selective substrates or inhibitors for mast cell and basophil derived proteolytic enzymes. Such substrates or inhibitors may inhibit triggering of target cells but will also inhibit the secondary activation which is probably due to the release of mast cell and basophil proteases. We have also found that similarly, competing sugars, (which were identified on the basis of proteins that interact with carbohydrate residues on either IgE or cells expressing cellular receptors) such as N-acetyl-D-glucosamin, a-methyl-mannoside, N-acetyl neuraminic acid, b-D-galactose, a-L-fucose, and lactose can inhibit the triggering of mast cells and basophils by interaction with lectins present in, for example, pollen or bacterial and viral agglutinins.

We have therefore successfully identified a number of substances which may be called antagonists and which may be responsible for inhibiting the activation of a mast cell-line or basophil cell-line.

According to a yet further aspect of the invention there is therefore provided a therapeutic composition comprising a substance containing a C-terminal lysine or arginine residue for the treatment of an allergic reaction.

According to a yet further aspect of the invention there is provided a therapeutic composition comprising a substrate or inhibitor, competitive or otherwise, of mast cell or basophil proteases for the treatment of an allergic reaction.

According to a yet further aspect of the invention there is provided a further therapeutic composition comprising a competing sugar that interacts with carbohydrate residues on either IgE or cells expressing lectin binding moieties for the treatment of an allergenic reaction.

In a preferred embodiment the sugar comprises N-acetyl-D-glucosamin or a-methyl-mannoside or N-acetyl neuraminic acid or b-D-galactose or a-L-fucose or lactose or other biologically active derivatives.

An embodiment of the invention will now be described by way of example only.

Figure 1

Histogram of the release of 5-hydroxytryptamine (5HT) from transfected cell-lines. Clones were incubated for 24 hours at 37 degrees C with Dex, o-dinitrophenol (DNP)-specific rat IgE or 4-hydroxy-3-nitrophenacetyl caproic acid (NIP)-specific human IgE (hIgE) in the presence of Dexamethasone (Dex) (10^{-8}M) and [^{3}H]-5HT. Cross-linking of receptor occupied IgE was effected with either DNP or NIP linked to human serum albumin (HSA) in the presence and absence of 5'(N-ethylcarboxyamido)-adenosine (NECA) (100 μM). [^{3}H]-5HT was measured after a 15 minutes incubation period and release was corrected for background and expressed relative to the total tritiated [^{3}H]-5HT incorporated.

Figure 2

A comparison of the release of [^{3}H]-5HT and β-hex-osaminidase. Transfected cells (H 2/2/C) were incubated with Dex (10^{-6}M), NIP-specific hIgE (1 μg/mL) and, where appropriate, [^{3}H]-5HT for 24 hours at 37 degrees C. The cells were washed and triggered with NIP-HSA in the presence of NECA (100 μM). The release of pre-loaded [^{3}H]-5HT (●) and endogenous β-hexosaminidase (O) were assessed. Each value is the mean of two tests; release was corrected for background.

Figure 3

Triggering of RBL-2H3 (H 2/2/C) transfectants following sensitization with serum from a bee-venom-allergic individual. Cells were incubated with the serum (1:10), Dex (10^{-6}M) and [^{3}H]-5HT. After washing and pre-incubation the cells were challenged with a range of concentrations of purified bee venom phospholipase A_2 (PLA_2). The percentage release of [^{3}H]-5HT (●) and β-hexosaminidase (O) were determined; data were corrected for background and normalized with respect to values obtained for triggering with hIgE-DNP (1 µg/mL) and NIP-HSA (100 ng/mL).

Figure 4

Transfected RBL-2H3 clones were incubated for 24 hours with or without serum (1:10 dilution) from a bee venom sensitive individual (EC) in the presence of 10^{-6}M dexamethazone and [^{3}H]-5-hydroxytryptamine. After washing, cells were challenged with antigen in the presence of 100µM 5' (N-ethylcarboxyamido)-adenosine (NECA).

[^{3}H]-5-Hydroxytryptamine was measured after a 15 minutes incubation period, release was corrected for background and expressed relative to the total [^{3}H]-5-hydroxytryptamine incorporated. For experimental details see Wilson, A. P. M., Pullar, C. E., Camp, A. M., and Helm, B. A., Human IgE Mediates Stimulus Secretion Coupling in Rat Basophilic Leukemia Cells Transfected with the Alpha Chain of the Human High-Affinity Receptor, European Journal of Immunology, 23:240-244, 1993, or alternatively, please see other references cited on Page 2 of this application.

Table 1

Parameters for the binding of iodinated [^{125}I] rat and human IgE to *parental and transfected clones.*

Clones were plated at 5 x 10^4 cells/well and incubated with Dex (10^{-6}M) for 24 hours at 37 degrees C. Parental cell-lines (RBL-2H3 intermediate secretors (I) and high secretors (H)) were incubated in the absence (A) and presence (B) of the steroid. Cells were incubated with a range of concentrations of [^{125}I] labelled rat or hIgE in the presence and absence of a 50-100-fold excess of unlabelled ligand (for experimental details see Wilson, A. P. M., Pullar, C. E., Camp, A. M., and Helm, B. A., Human IgE Mediates Stimulus Secretion Coupling in Rat Basophilic Leukemia Cells Transfected with the Alpha Chain of the Human High-Affinity Receptor, European Journal of Immunology, 23:240-244, 1993, or alternatively, please see other references cited on Page 2 of this application). Before counting, cells were washed and lysed, and the data were analyzed by the method of Scatchard.

nb = non binder; N = number of determinations

Table 2

Antigen-induced mast cell mediator release from RBL-2H3 cells in the absence of sensitization with antigen-specific IgE.

Parent RBL-2H3 cells or clones transfected with the α-chain of the human high-affinity receptor complex were plated out in 24-well plates at 2 x 10^5 cells/well as described previously in the citations referred to on Page 2 of this application. For the determination of antigen-induced mediator release, plates were incubated at 37 degrees C for 15 minutes, cooled on ice, the supernatant was removed, spun at 200 x g (1 minute)

before liquid scintillation counting to measure [3H]-5-hydroxytryptamine (5-HT) release. The ImmunoTech histamine enzyme immunoassay was used to quantify histamine release, and hydrolysis of toluene sulphonyl methyl ester was employed to monitor protease release (please see Wilson, A. P. M., Pullar, C. E., Camp, A. M., and Helm, B. A., Human IgE Mediates Stimulus Secretion Coupling in Rat Basophilic Leukemia Cells Transfected with the Alpha Chain of the Human High-Affinity Receptor, European Journal of Immunology, 23:240-244, 1993, or alternatively, please see other references cited on Page 2 of this application).

\+ 3-8% mediator release

++ 8-15% mediator release

+++ 15-25% mediator release

++++ 25-45% mediator release

ND Not Determined

Example 1

A method and corresponding assay system for determining the allergenic status of a given individual.

2×10^5 cells of RBL-2H3 transfected with the α-chain of the human high-affinity receptor for IgE were placed in at least one test well and incubated in a total reaction volume of 0.4ml buffer A or 24 hours at 37 degrees C with tritiated 5HT (1uCi/ml), in the presence of serum from an individual to be tested, a 2-100 fold dilution of human serum was used. (This serum may or may not contain antigen specific IgE corresponding to the allergen in the test method/kit depending upon the allergenic status of the individual.)

Following incubation, the cells were washed (2 x 1ml) with buffer A (120mM NaC1, 5mM KC1, 25mM PIPES, 1mM $CaC1_2$, 0.04mM $MgC1_2$, 5.6mM glucose, 0.1% BSA, pH7.4) and pre-incubated for a period of 10 minutes at 37 degrees C with buffer A (0.5ml) which was removed before challenge with buffer A (0.4ml) supplemented with the antigen in an amount of nanogram, microgram or milligram concentrations depending on the efficacy of the antigen source.

For the determination of antigen induced [^{3}H]-5HT release, the well was incubated at 37 degrees C for 15 minutes, cooled on ice, supernatant was then removed and spun at 2000g (1 minute) before liquid scintillation counting (2 minutes). The percentage release of [^{3}H]-5HT was calculated by the method of Siraganian and Hook (Manual of Clinical Immunology of the American Society for Microbiology Washington DC, 1980 Page 808). Histamine release was demonstrated employing the ImmunoTech histamine immunoassay kit.

(We have also determined the release of proteases from this cell-line and this was analyzed by the method of Hummel [Homodified Spectrophotometric Determination of Chymotrypsin, Trypsin & Thrombin, Canadian Journal of Biochem Physiol Volume 37, Page 1959].)

As mentioned, the ability of high-secretor variant clones to support mediator release through receptor-bound human IgE was determined either by pre-loading the cells with [^{3}H]-5HT or by measuring the release of endogenous β-hexosaminidase. Briefly cells were plated out and incubated for 24 hours at 37 degrees C, with [^{3}H]-5HT (if appropriate), Dex, o-dinitrophenol-(DNP-) specific rat IgE or 4p-hydroxy-3-nitrophenacetyl caproic acid-(NIP-) specific hIgE. The cells were washed with buffer A, pre-incubated for 10 minutes at 37 degrees C with the same buffer and challenged, in fresh buffer, with either DNP-HSA (DNP conjugated to human serum albumin) 50 ng/ml or NIP-HSA 10 ng/ml in

the presence of the adenosine analogue 5' (N-ethylcarboxyamido)-adenosine (NECA) 100 μM.

Properties of the transfected line

Following electroportation, transfection frequencies of 30 x 10^{-6} and 1.5 x 10^{-6} were obtained for RBL-2H3 intermediate secretors (I) and RBL-2H3 high secretors (H), respectively. On the basis of hFceR1α expression, three clones, namely I 5/3/C, H 2/2/C and H 7/1/A, were selected for further studies. Table 1 shows the relative number of receptors, following induction with Dex, demonstrating rat IgE and human IgE binding; rat IgE binds to the humanized receptor but with a 10-fold lower affinity than the natural ligand. As the interaction of hIgE with its receptor is an order of magnitude higher than the interaction of rat IgE with its receptor, the net result is that rat IgE engages hFceR1α with the same affinity as the rodent receptor. In addition to the data in Table 1, the rates of association and dissociation of rat and hIgE with I 5/3/C and rat IgE with the parental line RBL-2H3 (I), and the K_d values calculated from these on/off rates were consistent with published data.

A feature of this application is the ability of the transfected cell-line to support mediator release in response to cross-linking receptor-associated hIgE. Significant release required the presence of NECA (100 μM), at the cross-linking stage, in order to counteract the effect of Dex. This steroid decreases antigen-induced cell secretion but increases the responsiveness to NECA. It has been suggested that Dex down-regulates the expression of the G protein $G_{\alpha z}$ that may play a role in coupling the FceR1 complex to effector systems involved in mast cell exocytosis. Figure 1 shows the release of 5HT from transfected cell-lines following priming with either rat or human IgE and clearly demonstrates the effect of NECA. Simulation through hFceR1α gave consistently higher release than when degranulation was effected through the native rodent receptor complex.

Figure 2 shows a comparison between the release of β-hexosaminidase and preloaded [^{3}H]-5HT from H 2/2/C following hIgE-mediated, antigen-induced degranulation. The two assay systems compare reasonably well, although the former consistently yields lower values (7-10%) than [^{3}H]-5HT measurements.

An example of the use of the transfected cell-line for the measurement of antigen-specific IgE is shown in Figure 3, clone H 2/2/C was incubated with serum from a bee-venom-sensitive individual (EC) and challenged with bee venom PLA_2. A typical bell-shaped dose response curve for the release of β-hexosaminidase and [^{3}H]-5HT was observed when the IgE sensitized cell-line was challenged with increasing concentrations of PLA_2. Again the percentage release of β-hexosaminidase was less than that observed for [^{3}H]-5HT.

These results thus illustrate that the cell-line we have developed can be used, as we alone have realized, for the provision of a method and an assay system which can detect the allergenic status of an individual.

Example 2

A method and corresponding assay system for detecting the potential irritancy or allergenicity of chemicals.

2 x 10^5 cells of the RBL-2H3 cell-line transfected with the α-chain of the human high-affinity receptor for IgE were placed in at least one incubator well and incubated in a total volume of 0.4ml of buffer A (120mM NaC1, 5mM KC1, 25mM PIPES, 1mM $CaC1_2$, 0.04mM $MgC1_2$, 5.6mM glucose, 0.1% BSA, pH7.4) for 24 hours at 37 degrees C with [^{3}H]-5HT (1uCi/ml). (In this instance, in the presence of human serum and therefore in the presence of any antigen specific IgE.)

Following incubation, the cells were washed (2 x 1ml) with buffer A and pre-incubated for a period of 10 minutes at 37 degrees C with buffer A (0.5ml) which was removed before challenge with buffer A (0.4ml) supplemented with the irritant or antigen. The amount of irritant or antigen was in the range of nanograms, micrograms or milligrams depending on the efficacy of the irritant source.

For the determination of irritant or antigen induced [^{3}H]-5HT release, the well was incubated at 37 degrees C for 15 minutes, cooled in ice, supernatant was then removed and spun at 2000g (1 minute) before liquid scintillation counting (2 minutes). The percentage release of [^{3}H]-5HT was calculated by the method of Siraganian and Hook. Histamine release was demonstrated employing the ImmunoTech histamine immunoassay kit.

(We have also determined the release of proteases from this cell-line and this was analysed by the method of Hummel)

When the transfected cells were sensitized with the serum from a bee venom phospholipase A_2 sensitive individual (EC), mediator release could be demonstrated following challenge with purified bee venom phospholipase A_2 (mellitin free). A typical bell-shaped dose response pattern was observed when the sensitized cells were challenged with increasing doses of antigen. Control experiments, where non-sensitized cells had been incubated with the same concentration of antigen in the absence of sensitizing serum also showed degranulation of mediators, but this time mediator release increased in response to antigen concentration (see Figure 4)

Employing this cell-line, we were able to demonstrate that even in the absence of IgE several well defined allergens, (which in susceptible individuals give rise to an IgE response following the initial encounter) such as bee and vespid proteins, phospholipases, proteases from house

dust mites and fungal spores, lectins present in pollen and grain, latex-associated products and spermicides, and aspirin based drugs can trigger the release of substantial levels of mediators of the allergic response from these cells (see Table 2).

The above results indicate that, as we have realized, our cell-line can be used for determining the potential irritancy or allergenicity of a chemical or substance.

Example 3

In the development of a therapeutic composition for attenuating antigen-induced mediator response to allergenic stimulation the following method was used.

2 x 10^5 cells of the RBL-2H3 cell-line transfected with the α-chain of the human high-affinity receptor for IgE were placed in at least one incubation well in a total reaction volume of 0.4ml of buffer for 24 hours at 37 degrees C with [^{3}H]-5HT (1uCi/ml), in **either** the presence or absence of antigen specific IgE, that is either in the presence or absence of human serum (as above described) depending upon the nature of the allergic reaction to be attenuated. For example, where a composition was being tested or developed for its ability to attenuate an IgE mediated response such as asthma or hay fever, antigen specific IgE would be present, for example, in the form of human serum.

Following incubation, cells were washed (2 x 1ml) with buffer A (120mM NaC1, 5mM KC1, 25mM PIPES, 1mM $CaC1_2$, 0.04mM $MgC1_2$, 5.6mM glucose, 0.1% BSA, pH7.4) and pre-incubated for a further period of 10 minutes at 37 degrees C with buffer A (0.5ml) which was removed before challenge with buffer A (0.4ml) supplemented with a pre-selected antigen (in an amount as afore described) **and** the therapeutic composition or the antagonist.

For the determination of antigen induced [^{3}H]-5HT release, the well was incubated at 37 degrees C for 15 minutes, cooled on ice, supernatant was then removed and spun at 2000g (1 minute) before liquid scintillation counting (2 minutes). The percentage release of [^{3}H]-5HT was calculated by the method of Siraganian and Hook. Histamine release was demonstrated employing the ImmunoTech histamine immunoassay kit.

(We have also determined the release of proteases from this cell-line and this was analysed by the method of Hummel)

In the instance where the therapeutic composition acted as an effective blocker of the antigen-induced allergic response, there was a significant reduction in [^{3}H]-5HT release.

Activated mast cells and basophils also secrete at least three proteases, the physiological function of which is unknown. These enzymes are serine endoproteases with a trypsin-like specificity. We have recently shown that after secretion following an immunological or non-immunological stimulus, proteases released from the activated RBL cell-line can induce mediator secretion from cells of their kind. This secondary burst of the release of inflammatory mediators can be attenuated by the inclusion of protease inhibitors or substrates for serine proteases like the synthetic substrate p-toluenesulphonyl-L-arginine methyl ester (TAME) or the human IgE-derived pentapeptide (HEPP) in the medium bathing the cells. This observation provides a self-evident explanation for the observed therapeutic effect of administration of HEPP to patients suffering from eg allergic rhinitis, initially attributed to be due to competitive inhibition of the IgE/FceR1 interaction, but later held untenable.

It will be understood that the therapeutic composition identified using the above method may be manufactured in any saleable form such as a pill, capsule, lozenge, tablets, medicine, infusion, ointment, nasal spray, inhalant or any other known means of manufacturing a medicament.

It will be understood that although the examples have been described by reference to an immuno-radioactive method, it is within the scope of the invention to deploy a colourimetric technique, or proteolytic assay, or immunological assay, or antibody assay, or indeed any other standard technique of assay such as a measurement of membrane potential for determining the response of a mast cell-line or basophil cell-line to a given allergen or antagonist under the above specified conditions.

CLAIMS

1. A method for determining the allergic status of an individual comprising:

a. exposing a cell-line, which is a secretor variant of mast cell or basophil lineage and is transfected with a moiety capable of binding human IgE, to a sensitizing agent;

b. challenging the cell-line with at least one allergen; and

c. determining the release of mast cell or basophil mediators in response to said challenge.

2. A method according to Claim 1 wherein said mast cell-line is an RBL-2H3 cell-line which is transfected with the α-chain at least of the human high-affinity receptor for IgE.

3. A method according to Claim 1 wherein said sensitizing agent is human IgE.

4. A method according to any preceding Claim wherein said cell-line is pre-incubated in a solution containing a radio active marker.

5. A method according to Claim 4 wherein said marker is tritiated histamine.

6. A method according to Claim 4 wherein said marker is ^{14}C arachadonic acid.

7. A method according to Claims 1-3 wherein spectrophotometric means is used to determine release of mediators.

8. A method according to Claims 1-6 wherein said release of mediators is determined using an immunoassay technique.

9. An assay kit for determining the allergic status of an individual comprising:

a. a cell-line which is a secretor variant of a mast cell-line or a basophil cell-line and is transfected with a moiety capable of binding human IgE;

b. a test allergen; and

c. means necessary to determine the absence or presence of an immune response.

10. An assay kit according to Claim 9 wherein the cell-line is an RBL-2H3 cell-line which is transfected with the alpha-chain at least of the human high-affinity receptor for IgE.

11. An assay kit according to Claims 9 or 10 wherein there is further provided a pre-determined amount of radio active marker.

12. An assay kit according to Claim 11 wherein said marker is tritiated histamine.

13. An assay kit according to Claim 11 wherein said radio active marker is arachadonic acid.

14. An assay kit according to Claims 9 or 10 wherein there is provided a chromogen means for measuring release of mediators.

15. An assay kit according to Claims 9-13 wherein there is provided immunoassay means for measuring release of mediators.

16. A method for determining the potential irritancy or allergenicity of a pre-selected substance comprising:

a. exposing a mast cell-line and/or basophil cell-line to said substance in the absence of a sensitizing agent; and

b. then determining the release of mast cell and/or basophil cell mediators in response to said exposure.

17. A method for determining the potential irritancy or allergenicity or a pre-select substance comprising:

a. exposing a cell-line, which is a secretor varient of mast cell or basophil liniage and is transfected with a moiety capable of binding human IgE, to said substance in either the absence or presence of a sensitising agent; and

b. then determining the release of mast cell and/or basophil cell mediators in response to said exposure.

18. A method according to Claim 16 or 17 wherein said cell-line is a high-secretor variant.

19. A method according to Claim 16, 17 or 18 wherein the cell-line is a secretor variant of RBL-2H3.

20 A method according to Claims 16-19 wherein said cell-line is pre-incubated with a marker.

21 A method according to Claim 20 wherein the marker is tritiated 5-hydroxytryptamine or histamine.

22. A method according to Claim 20 wherein the marker is ^{14}C arachadonic acid.

23. A method according to Claims 16-19 wherein the method comprises exposing said cell-line to a chromogen which changes colour as a result of the presence of an immunogenic reaction.

24. A method according to Claims 16-22 wherein the method comprises exposing said cell-line to an immunoassay means for measuring release of mediators.

25. A therapeutic composition comprising a substance containing a C-terminal lysine residue for the inhibition of mediator release in an allergic reaction.

26. The use of a substance containing a C-terminal arginine residue for the manufacture of a medicament for the inhibition of mediator release from activated mast cells or basophil cells in an allergic reaction.

27. The use of a substance comprising a substrate or inhibitor of a mast cell of basophil cell endoprotease secreted in response to activation of the mast cell or basophil cell for the manufacture of a medicament for the treatment of an allergic reaction.

28. A therapeutic composition accordinfg to Claim 27 wherein said protease is a serine endoprotease.

29. A therapeutic composition according to Claim 27 wherein said substrate is p-toluenesulphonyl-L-arginine methyl ester (TAME).

30. A therapeutic composition according to Claim 27 wherein the inhibitor is a human IgE-derived pentapeptide (HEPP).

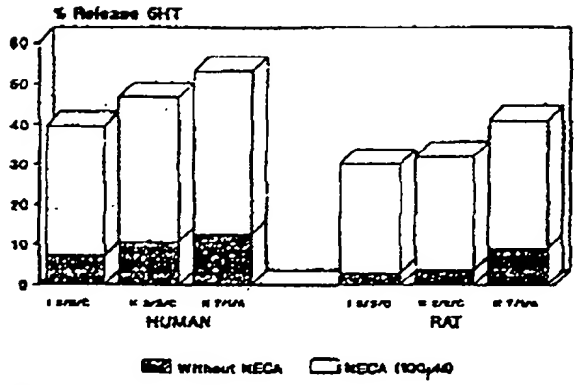

Fig.

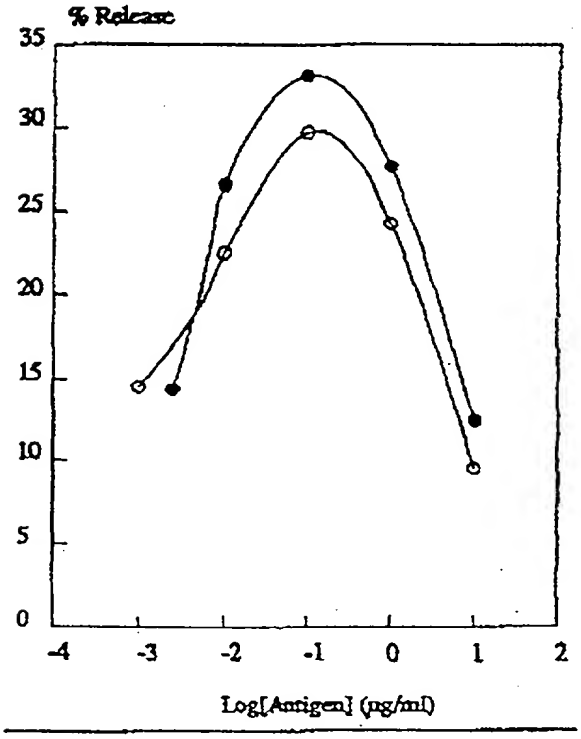

Fig. 2

AMENDED SHEET

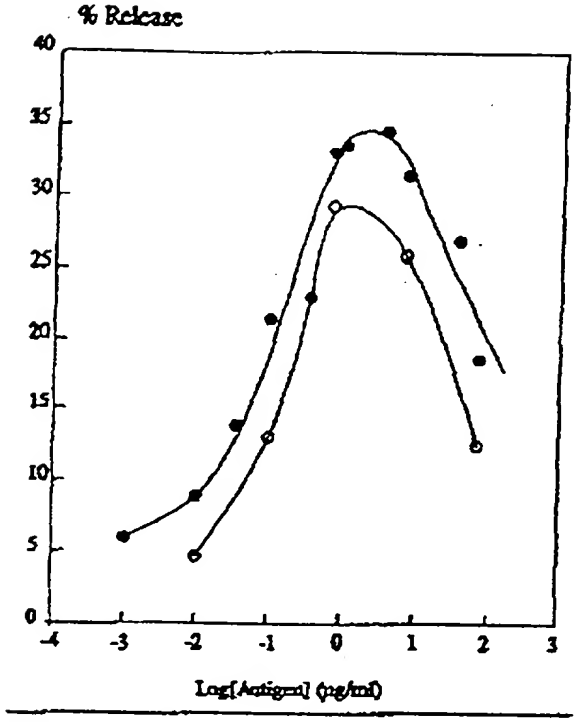

Fig. 3

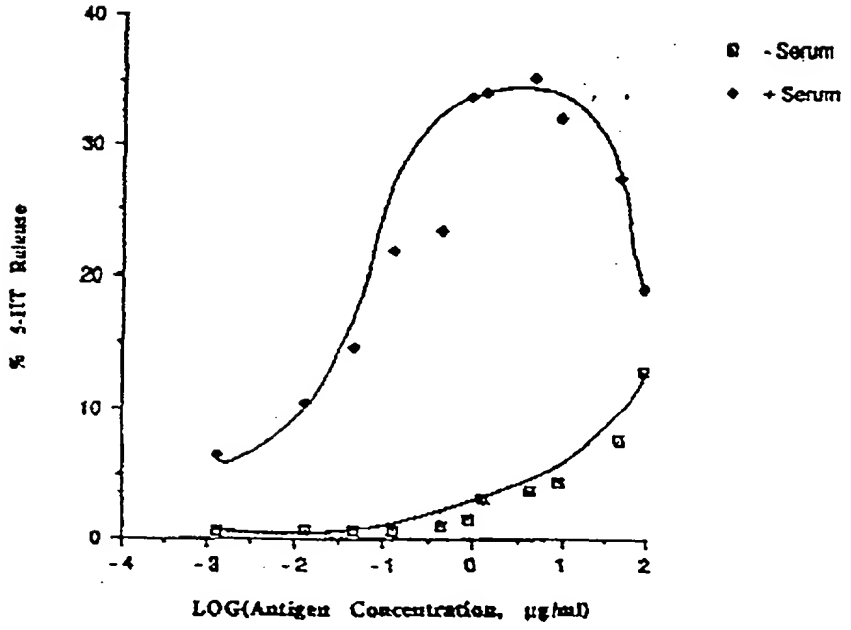

Figure 4

TABLE 1

Clone	Ligand	K_a (nM)	Receptors/cell ($\times 10^{-3}$)	N
RBL-2H3 (5)	hIgE	nb	0	>10
	rIgE (A)	14.0 ± 0.4	10.6 ± 0.7	3
	rIgE (B)	13.5 ± 0.2	10.7 ± 0.8	2
RBL-2H3 (10)	hIgE	nb	0	>10
	rIgE (A)	12.7 ± 0.4	8.3 ± 0.5	3
	rIgE (B)	13.8 ± 0.7	8.1 ± 0.6	2
I 5/3/C	hIgE	2.3 ± 0.03	2.2 ± 0.04	2
	rIgE	15.1 ± 0.8	17.3 ± 0.4	2
H 2F2/C	hIgE	2.2 ± 0.04	1.0 ± 0.1	2
	rIgE	10.4 ± 0.5	11.0 ± 0.8	2
H 7F1/A	hIgE	2.4	0.7	1
	rIgE	14.0 ± 0.6	0.1 ± 0.8	2

Table 2

Antigen	Mediators Measured: 5-HT	Protease	β-Hexosaminidase
Venom, bee/wasp (1% suspension)	++++	+++++	++++
Phospholipase A2 (bee venom), 1μg/ml	+	+	+
Phospholipase C (*B. cereus*)	++++	++++	ND
House Dust Mite extracts, (freshly prepared) 1% suspension	+++	+++	ND
House Dust Mite extracts, commercial sources, 1% suspension	-	+/-	ND
Condom extract (1:400) (*Hevea brasiliensis*)	++	++	ND
Aspirin (25mg/ml)	+++	+++	ND
Influenza virus (5% suspension)	+	+	ND
Herpes simplex virus (5% suspension)	+	+	ND

AMENDED SHEET

COMBINED DECLARATIC OR PATENT APPLICATION AND POWER ATTORNEY

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER: HELM ET AL PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No 08/446,760

on July 21, 1995.

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/GB93/02430

on 25, November 1993

and was amended under PCT Article 19

on November 30, 1994 (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above

I acknowlege the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Great Britain	92 249 56.4	28. November 1992	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

PTO 1391 (REV. 10-83) Page 1 of 2 U.S. DEPARTMENT OF COMMERCE Patent and Trademark Office

(January 1991)

Combined Declaration For Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER: HELM ET AL PCT

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowlege the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(List name and registration number)*

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THOMAS M. GALGANO, ESQ., REG. NO. 27,638
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
X Birgit Helm	X [signature]	[signature]
DATE: 28/04/98	DATE: 3/4/98	DATE: 14/04/98

Combined Declaration For Pater ...plication and Power of Attorney (Continu...
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER: HELM ET AL PCT

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PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE & ZIP CODE/COUNTRY:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
X Christine E Pullar	X	
DATE: X 6/4/98.	DATE: X 8/4/98	DATE